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# THE VENEREOLOGY LABORATORY

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The laboratory should play a part in all types of veneral diseases; there is none of them for which it is not to be called on.

The venereal diseases are "infectious" diseases, whose causal agent it is important to detect at the time of clinical diagnosis and whose disappearance after treatment should be verified.

When the diagnosis is or appears obvious (acute gonococcia or syphilitic chancre), it is not justified to prescribe a treatment before having the laboratory's confirmation. After treatment, the laboratory contributes indispensable elements to diagnosis of cure.

In the case of syphilis, serological examinations repeated after antibiotic treatment are an imperative which cannot be ignored without committing a professional error.

These diseases, transmitted by sexual contacts, have a social incidence which every practicing physician knows. Here the laboratory plays a preponderant role, especially in cases in which the partner or partners present little or nothing in the way of clinical signs (gonococcia in women; trichomoniasis, candidiasis in men; serological syphilis of men or women).

## I. THE GONORRHEAS

### A. Manner and Points of Sample-Taking

1. Precautions in Sample-Taking

The quality of sample-taking is one of the essential factors for success with regard to gonorrhea. It is important to keep two fundamental principles in mind:

-- most agents of gonorrheas are fragile germs;

-- in men, it is best to take samples in the morning.

The first principle applies to isolation of the gonococcus and the Trichomonas. The best conditions for sampling are those of the laboratory, with the sample inoculated directly into the selective media. Intermediary media for transport are only makeshifts, and are to be used only in exceptional cases, when circumstances prevent sampling at the laboratory. Transport media are poor ones, permitting survival of the germs without offering the nutritive elements necessary for their metabolism. Under these conditions, success is a function of the duration of the trip in the intermediary medium and of the vitality of the strain isolated. As will be realized easily, strains adapt themselves more or less well to the transport medium, and in certain cases, the transplantation will be a failure.

Except in the case of acute gonococcia, in which large quantities of the germs are in the pus and it is not easy to detect them, there is the greatest chance of success when the sampling is done in the morning, before any urination. Reactivation by alcohol (beer, alcohol), the day before sampling, is sometimes useless.

2. Sampling Method

-- Taking of pus sample with platinum needle, for direct search for gonococcus; film thin and on sufficiently large plate surface, so as to separate well the plaques of multinuclear cells and urethral cells; drying in air.

-- Sample taken with slightly sharp spatula for search for viral inclusions or PPLO (pleuropneumonia-like organisms). The object is to extract from the roof of the urethra any cells which may be parasitized. A good sample-taking must not tap any blood from the urethra; a plate containing too many red blood corpuscles makes the reading difficult, and in the extreme, impossible. "Round" spreading on plate. Fixation by methyl alcohol.

-- Sample-taking with platinum needle for trichomonas. Here, two plates are necessary -- one prepared for search for gonococcus, the other for fresh-state examination under microscope against dark

ground. For the latter, a slide is prepared on which a drop of lukewarm physiological serum (37°) has been deposited. The secretion taken from the physiological serum is mixed; a glass cover is placed over the sample; and examination is done immediately.

-- In case of deep-seated injury (posterior urethra, prostate), the sample may be taken:

-- after massage of the posterior urethra from back to front, up to the meatus;
-- by taking a filament from the first spurt of urine;
-- by residue from centrifugation of the first spurt of urine;
-- after massage of the prostate (in the case of cavities).

-- For the gonococcus culture, the pus is taken from the secretion with a cotton swab of rattan, sterilized under dry heat (100°, 45 minutes). This sample is immediately spread on the selective medium whose plate has previously been heated in an oven (37°).

-- For the Trichonomas vaginalis culture, the same equipment is used, but the swab is left in the culture medium (liquid medium).

3. Sampling Points

The anatomical lairs of the agents of the gonorrheas are more numerous than is usually believed:

a. In man:

The anterior urethra, to which there is too often a tendency to limit gonorrhea in men. It is, of course, the preferred locus of acute infections (gonocaccia, urethritis of commonplace pyogenics [sic], trichomonasis) or viral diseases (chlamydozoon, PPLO).

The posterior urethra may also be the seat of gonorrheal lesions. The urethral secretion is minimal (meatus stuck closed), or even absent. In this case, massage of the urethra, in the morning before urination, is very valuable. If this does not induce any secretion, a urinary filament should be sought; or in its absence, the residue from centrifugation of the urine should be examined.

The prostate (especially in cases of prostatic cavities) should furnish material for the sample, after massage of the gland and of the urethra, along all the accessible length.

Cooper's paraurethral glands may also be infected; exploration

of them will sometimes reveal a zone in activity.

Anal gonorrhea, due mainly to the gonococcus, is more frequent than is believed, and should not go unrecognized. The sample is made at the anus, in the same manner as with the urethra.

b. In women:

-- Urethra: Here, the sample is taken after massage of the canal by intravaginal finger, which draws the secretion from back to front up to the meatus.

-- Pus in the Skene glands, very often infected, is made to come out by compressing the gland between two fingers.

-- Bartholin glands: Same principle as for the Skene glands.

-- Vagina: The gonococcus is never found here. Here one looks for Trichomonas, yeasts, commonplace germs. It is important to take the sample by using a dry speculum (without soap or lubricant). In view of the disagreeable character of this operation for the patient, one may without inconvenience introduce into the vagina a longish tweezer arm and take the sample from the material collected on the tweezer.

-- Neck of uterus: Sampling is done here mainly to look for gonococcus. It should be endocervical, and done with the aid of a tampon on a rattan swab.

-- Anus: Sampling presents no particular problems.

## B. The Agents of the Gonorrheas

### 1. Gonococcus (Neisseria gonorrhoeae)

This is a Gram negative Diplococcus, easy to recognize in acute male urethritis by its characteristic frog spawn grouping, and its intracellular location.

On the other hand, it is more difficult to identify in chronic infections (men and women), in which the masses are less thick, in which the intracellular character tends to disappear, and in which the germ is more readily extracellular.

In these cases, diagnosis cannot be based on a simple direct examination (Gram coloration); it is indispensable to use a culture.

Without going into the technical details, it may be said that:

-- Whatever medium is chosen, the culture should be done in a carbonic atmosphere (10%) for 48 hours.

-- In order to recognize the colonies of gonococci, an oxidase reaction is done by pulverizing a solution of p-phenylenediamine hydrochloride on the surface of a culture dish. The colonies of gonococci are oxidase positive -- i.e., in a few minutes they take on a pink, then violet, then black coloration.

-- An oxidase + colony is withdrawn, and its germs are examined after Gram coloration. Gram negative cocci should be revealed.

Usually, the laboratory's role stops here. However, it should be understood that:

-- not all the Gram negative, oxidase positive cocci are necessarily gonococci, and this explains the therapeutical failures which occur. The gonococcus belongs to the family Neisseria, which comprises a whole population of non-pathogenic Gram negative, oxidase positive cocci.

-- In case of doubt about the authenticity of a gonococcus, it is necessary to make an identification of the germ on mediums containing sugar. Among the Neisseria, the gonococcus is the only one to produce the pattern:

glucose + lactose + maltose -

The ocular complications of gonococcia (purulent ophthalmia of newborn) have practically disappeared since introduction of the prophylactic methods employed at birth. The same holds for articular complications since introduction of antibiotic treatment. At most, a metritis of the body can still be seen, or a gonococcic salpingitis. In this case, taking a sample from the purulent endocervical mucus should ensure diagnosis.

Finally -- and to conclude with regard to the principal agents of the gonorrheas --, we say that in some cases, one may be led to study the sensitivity of the germ to different antibiotics. These cases are rare if the extent of the antibiotic arsenal is compared with the fragility of the Neisser Diplococcus.

2. Trichomonas vaginalis

This germ long neglected, has in the last few years received attention which is well merited if one considers not the seriousness of the infection which it causes, but the disagreements which it engenders.

The Trichomonas, a protozoan of large size, lives in the mucous cavities. Bacteriological diagnosis of it does not pose any problems. Its form, the mobility of its flagellae, and its undulant membrane suffice to characterize it. This locomotive apparatus enables it to move about fast.

The best bacteriological method for examining a suspect pathological product is study of the secretion under the microscope against a dark ground, on the strict condition that this examination be made as soon as the sample is taken. Under poor conditions, five minutes are enough for the Trichomonas to lose its movements and its form, and thus to become unrecognizable.

Examination after coloration (Giemsa) makes possible the study of samples taken far from the laboratory, and in certain cases can be useful.

Culture of Trichomonas vaginalis necessitates a medium well adapted to the germ's requirements. In certain cases (in particular, chronic urethritis in males), it can make possible a diagnosis which is difficult by another method.

In men, trichomoniasis is limited to the urethra; in women, to the vagina and the urethra.

3. Candida albicans

The genital tracts frequently harbor yeasts (Candida). Among them, only Candida albicans is pathogenic (vaginitis, urethritis). Hence the laboratory should not be content with incomplete diagnosis of "yeasts," but should make an identification of the germ.

This search is based on the production by Candida albicans of chlamydospores which suffice to characterize it. Chlamydospores require special mediums, but of simple composition (rice gelose, for example), in order to appear.

4. The Commonplace Germs

These germs cause urethrites or vaginites quite frequently. These lesions perhaps do not have a place in this article, for they are not necessarily transmitted by sexual contact. Usually they are germs lodged in the vagina or in the urethra (staphylococci, colibacilli, etc.) and reactivated under various influences. However, it is important to know of their existence.

5. The PPLO Viruses, "L" Forms

The virus of amicrobic urethritis is the oculo-genital chlamydozoon. This is an organism close to that of trachoma. The viral urethritis may be isolated or enclosed in a complex urethro-conjunctival-synovial syndrome (Fiessinger-Leroy-Reiter). This is mentioned as a reminder that one should not neglect to take a conjunctival sample, technically analogous to the urethral sampling (see above) in case of urethritis-conjunctivitis association.

The oculo-genital chlamydozoon colorated by the Giemsa method presents itself in the form of groups of intraprotoplasmic inclusions. The search for them is delicate, because of the great rarity of parasitized cells. The extracellular viral formations must be absolutely typical in order to be identified as this chlamydozoon. It must be understood that a large number of artifacts can be mistaken for viruses.

The oculo-genital chlamydozoon does not grow in the usual mediums. It passes through the bacteriological filters.

The pleuropneumonia-like organisms: This designation encompasses organisms of various origins. Christened thus because of their morphological similarity to the pleuropneumonia organisms, they comprise on the one hand germs of the pleuropneumonia genus, and on the other, forms of microbic mutation. Under certain influences, a germ may lose its conventional morphology, and in the course of a poorly understood cycle fix themselves in the aspect of "L" forms.

Like the viruses, the PPLO's have been found in certain amicrobic urethrites, and in the course of the urethro-conjunctival-synovial syndrome.

The PPLO's and "L" forms grow on enriched gelose, but pass through the bacteriological filters.

6. The Amicrobic Urethrites As Such

The role of the laboratory, like that of the physician, is confounded here with that of the psychotherapist. These urethrites are specific to men afflicted with anxiety; they are accompanied by a symptomatology which is richer as the subject is more advanced.

C. Practical Conclusions

1. When Can Diagnosis of Gonorrhea Be Made?

-- Incubation of the gonococcus is four days on the average, but authentic gonococci have been found from the second day after the infecting relationship. This incubation can be lengthened (in the case of delayed antibiotic treatment, for example), and a urethritis whose incubation is from eight to ten days cannot be considered a priori as non-gonococcic.

-- In case of trichomoniasis or moniliasis, the details of incubation are not very precise. A negative sample is not sufficient to reject the diagnosis; the examination must be repeated at an interval of several days. In women, the period at the end of the menses is the most favorable.

-- Same observation for the other agents.

2. When Should Cure Be Checked?

-- After total elimination by the organism of the medications used for cure. As a general rule, the period is from three to four days, except for the long-acting penicillins.

-- A cure can be confirmed only after two negative examinations (cultures), made at an interval of four days.

## II. SYPHILIS

The laboratory's part in syphilography is either to detect the pale treponema (PT), or to study the antibodies circulating.

A. Detection of Pale Treponema

The PT can be detected in any cutaneous or mucous lesion, as

well as in the tributary ganglia in the region of a chancre. In exceptional cases, it has been found in cephalorrachidian liquid.

The serum from a chancre or mucous plate cultures is teeming with PT's. To bring them out, a few precautions should be taken:

-- Make sure that the patient has not treated his lesion with antiseptic or antibiotic salves;

-- Scarify the surface of the chancre, allow to bleed, and collect the serous fluid which wells up after the blood is stopped;

-- If no PT's are found by this method, wash the surface of the chancre with cotton soaked in alcohol at 96°; wait a few minutes; then examine the serum again;

-- If the result is still negative, puncture the ganglion corresponding to the chancre, if it is accessible;

-- Do not exclude diagnosis of syphilitic chancre until after a second examination made 48 hours later;

-- Do not exclude diagnosis of syphilis until after serological surveillance of the patient for two or three months.

The search for the PT is made immediately after the sampling, between plate and glass cover, under microscope against dark ground. The coloration methods are to be rejected.

B. Study of Serology

The role of the laboratory is to make a diagnosis of serological syphilis, and to follow the progress of the antibody in a syphilitic. In the former case, qualitative serological reactions are involved; in the latter case, quantitativ reactions.

1. The Serological Techniques

These are of two types: (a) the non-specific antigen reactions (hemolysis-flocculation); (b) the specific antigen reactions.

a. The Non-Specific Antigen Reactions

It is not our purpose to consider here the ancestors of our present hemolysis reactions: Does one remember the reactions of Porges

and Maier, Yamanouchi, Fleischman, Calmette, Massol, Sachs, Desmoulieres, and so many others? To the long list of forgotten reactions we can add those of Porges, Klausner, Foix and Marcel Bloch, Georgi, Dreyer and Ward, the sigma reactions of Vernes, Meinicke, Kahn, etc. This improbable "technorrhea" broke out in France and abroad during the first third of our century.

Each new reaction, by increasing the already great confusion, seemed to make syphilis a disease different from the others.

These reactions, of a rather low quality and subject to numerous errors, had in their day the merit of being the only elements for diagnosis of latent syphilis and for surveillance of treated syphilitics. For this reason alone, they have a right to be respected in our memory.

Serious serology of syphilis began with the discovery of cardiolipid in 1941, which was to be the basis of most of the present antigens.

There are two types of reactions in serology -- the hemolysis reactions and the flocculations reactions.

1). The Hemolysis Reactions

These reactions seek to visualize the presence of antibody in a serum, by the modifications produced in an immune complex having no relation with syphilis. The modes of carrying out these reactions are the following:

-- Placing in contact cardiolipidic antigen and the serum to be examined, in the presence of a previously determined quantity of complement;

-- Period of sensitivation of the complex making possible fixation of the complement (in cases in which the serum examined contains antibody);

-- Introduction into the reaction of the detecting complex, in which the antigen is represented by red corpuscles of sheep and the antibody by anti-sheep rabbit serum.

When the serum to be examined contains antibody, the complement is used in the formation of the immune complex. There is no modification at the time of the introduction of sensitized red corpuscles of sheep. On the other hand, if the serum to be examined does not contain antibody, it will not form an immune complex; the complement will remain free to join the second complex and produce hemolysis from it.

Kolmer specified the best conditions for carrying out this reaction: use of small quantity of red corpuscles (2%), two units of complement, extended fixation in cold.

The antigen is cardiolipid; this is a salt of a complex phosphatic acid isolated from the cardiac muscle. When it is used alone, its serological activity is very low. On the other hand, its activity is considerably increased by the addition of serologically inert substances -- lecithin and cholesterol -- in suitable proportions. Cardiolipid in vivo has no antigenic properties; whence the justification of Wassermann's term "hapten," which should be applied to it regularly.

2). The Flocculation Reactions

Their principle is based on the fact that the antigen precipitates in contact with the serum of a syphilitic subject. At present, only cardiolipidic antigen is used for the flocculation reactions. Many techniques are proposed, but we shall take only Kline's technique, the most widespread one.

b. The Specific Antigen Reactions

These reactions use the agent of the disease itself, the pale treponema, as the antigen.

1). The Treponemal Immobilization Test (TIT)

The principle of the Nelson reaction is simple. A suspension of pale treponemas surviving in a synthetic medium serves as antigen. When serum from a syphilitic subject and fresh guinea pig serum is added to this suspension, the pale treponemas immobilize. This reaction, done for the last 14 years, has showed no failure. Its sensitivity is very high, its specificity very narrow, and consequently, its reproducibility is perfect. We shall pass over the technicalities of carrying out the TIT and discuss its principal qualities at somewhat greater length.

2). The Specificity

The specificity of an immunological reaction is based on the specificity of the antigen in relation to the disease, of the antibody in relation to the pathogenic agent, and finally, of the method which detects this antibody once it is joined to the antigen. In the case of the TIT, the antigen itself is the agent of infection.

The specificity of the antibody (immobilisin) remains a begging of the question; to our knowledge, however, it has never been detected at a significant rate in diseases other than syphilis or closely related treponematoses (yaws, bejel, pinta).

A number of cases have been published showing partial immobilization in vaccinated or malarial subjects.

These results call into question more the specificity of the technique used than that of the reaction. In effect, all tests aimed at reproducing these results have remained fruitless. If, for such serums, the reaction had been done several times, on the same or different samples, by several laboratories, the technical error would have become obvious.

Specificity of antigen and of antibody are intangible immunological facts which the biologist limits himself to observing. On the other hand, the same is not true for the specificity of the method which is a problem. The value of the results depends on this specificity. It is a direct function of the quality of the technique used. It would do no good to have specific antigen and antibody if the method were defective. Any technical flaw causes loss of specificity of the reaction, and hence, its reproducibility.

Nelson, by experience, set the maximum tolerated for non-specific immobilization at 20%. Above this, the presence of immobilisin in the serum can be asserted. If, for any technical reason whatsoever, this non-specific immobilization figure were increased, the results would be all the more vitiated.

The reproductivity of the TIT is indisputable.

The use of a suitable technique eliminates fluctuations of great amplitude (0% to 100% and 100% to 0%), which can only be errors of handling. Oscillations of small amplitude fall in the zone of partial immobilizations.

If we consider the two large classes of serums for which a TIT is requested, the cases for diagnosis and the cases for serological surveillance, we observe that the partial immobilizations are found practically only in the second class -- serological surveillance.

3). Immunofluorescence

It seems to us that this reaction, presently unknown in France, is one of the most interesting. Developed by Coons in 1942, it is based on the fact that one can color a globulin without modifying its

antigenic properties. Hence, one can easily see the principle of the reaction. first, "tag" an antibody globulin with a fluorescent chemical agent and place the antibody thus marked in contact with a preparation containing antigen; next, reveal the immunological complex by examination of the preparation in ultraviolet light.

This process, called the direct process, was the first described. It has the disadvantage of requiring a collection of tagged antibodies in a number equal to the antigens sought. It is for this reason that it must yield to the indirect process (called also the layer method). This process requires only a single fluorescent antibody for all the antigens to be studied.

This antibody is a solution of fluorescent rabbit globulins which are anti-human gamma globulin. One proceeds in the following manner:

-- fixation of the preparation;
-- formation of the first immunological complex by deposit of the non-fluorescent specific antibody;
-- washing to eliminate excess antibody;
-- formation of second immunological complex by deposit of fluorescent anti-globulin serum;
-- washing to eliminate non-fixed anti-globulin;
-- examination in ultraviolet light.

With this technique, the specific antibody plays the part of antibody in the first complex, and antigen with respect to the second. This is the process which we have applied to search for antibody in the serum of syphilitics.

The sensitivity of the reaction is theoretically very great, since one can detect the antigen-antibody complex practically in the molecular state. The sensitivity of the indirect method makes it possible to detect about ten gammas of antibody.

The elements which we presently have are insufficient for making a definitive judgment on the immunofluorescence reaction applied to syphilis. However, from the point of view of specificity and sensitivity, it seems very close to the TIT; its execution is infinitely more simple, and therefore it may some day be a rival of the TIT to be reckoned with.

2. Serological Problems

a. Detection of Antibody

(a) The reagin -- antibody detected by the cardiolipidic reactions --

is an antibody of rapid appearance, six weeks on the average after the infecting contact. It is always present during secondary syphilis; it is in this stage that it reaches the highest concentrations. In the course of tertiary syphilis, it is not rare to see it disappear spontaneously. After suitable treatment of the disease, the reagin disappears in almost all cases.

(b) The immobilisin is the antibody detected by the Nelson test (two to three months after the infecting contact). It persists throughout the disease, with the concentration simply fluctuating. The highest concentrations are observed in the course of nervous tertiary syphiles. This antibody disappears after treatment insofar as treatment comes at a relatively early period in the disease ($\mathcal{E}$, $\mathcal{E}_s$). Its later disappearance is hypothetical.

(c) The antibody detected by immunofluorescence is probably different from the preceding. It is very rapid in appearance (in some cases we have been able to detect it only a few days after appearance of the chancre). Like the immobilisin, it persists throughout the disease. The concentrations are very high in the course of secondary syphilis (1:196,000 in certain of our observations). Its disappearance after treatment is not yet very well defined, since there has not been sufficient perspective for judging.

Hence the distribution of antibodies in the course of untreated syphilis can be summed up schematically as follows:

| | Reagin | Immobilisin | Immuno-fluorescence |
|---|---|---|---|
| Primary syphilis | - or + | - rarely + | + or - |
| Secondary syphilis | + | - or + | + |
| Latent syphilis | + or - | + | + |
| Tertiary syphilis | + or - | + | + |

The search for syphilis (in the presence of a lesion or for any other cause) is customarily made by means of the cardiolipidic reactions (Kline and Kolmer). It is a reassuring indication if they are negative, although this cannot exclude syphilis (possibility of latent or tardy syphilis with negative serology). If they are positive, this is not a strict proof of syphilis (possibility of erroneous positive reactions). In our judgment, the search for syphilis by cardiolipidic reactions alone is not sufficient in all cases. It should be complemented by the treponemal immobilization reaction.

Cardiolipidic reactions may be sufficient in cases in which they

produce entirely positive hemolysis and flocculations reactions on two occasions. In cases where the reactions are dissociated, or negative, the TIT should be used. We recall in passing that the TIT is positive in yaws and bejel, which are due to treponematoses serologically identical with the PT. In France, this particularity of the TIT does not pose any problems.

The place of immunofluorescence in diagnosis of syphilis still remains to be made definite, but it seems certain that henceforth it should fall between cardiolipidic serology and the TIT, because of its sensitivity and its specificity.

In the course of treated syphilis, the interest of serodiagnosis lies exclusively in the quantitative study of antibodies. The fluctuations in concentration of the reagins, immobilisin or immunofluorescence are the best criterion for effectiveness of the treatment.

In conclusion, after this brief listing of the veneral diseases (we have not discussed the soft chancre, although it may be seen in exceptional cases), we should note the preponderant place of the laboratory: for diagnosis, on the one hand, but mainly as a check on the effectiveness of a treatment and as furnishing a criterion of cure.